CRYSTAL STRUCTURE OF AN ENZYME INVOLVED IN THE BIOSYNTHESIS OF ISOPRENOIDS:

4-diphosphocytidyl-2C-methyl-D-erythritol kinase from *E. coli*, a potential drug target.

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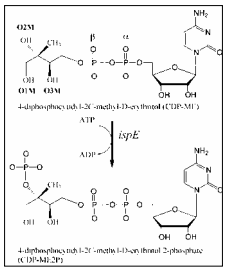
Isoprenoids are a diverse family of compounds consisting of isoprene units (five-carbons units) and are involved in many biological functions such as electron transport, hormone based signaling, programmed cell death (apoptosis), also they provide structural components of cell membranes **[1]**. In contrast to mammals, some pathogenic agents such as those responsible for serious human disease including leprosy, malaria, bacterial meningitis, tuberculosis and certain types of pneumonia **[2]** use the non-mevalonate pathway to synthesis those compounds. If we could disrupt this pathway, it might provide the first step in the development of a broad-spectrum antimicrobial agent. With this in mind, we solved the structure of the 4-diphosphocytidyl-2C-methyl-D-erythritol kinase (CDP-ME kinase). The resulting model reveals information as to the specificity and the catalytic mechanism of the enzyme.

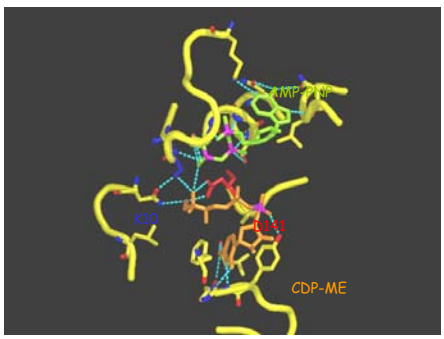
Reaction catalyzed



CDP-ME kinase catalyses the transfer of the ATP γ-phosphate to the O2M position in the 4-diphosphocytidyl-2C-methyl-D-erythritol , its substrate.

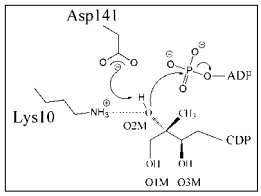
ADP and 4-diphosphocytidyl-2C-methyl-D-erythritol-2-phosphate are then released.

Detailed active site



Catalytic mechanism

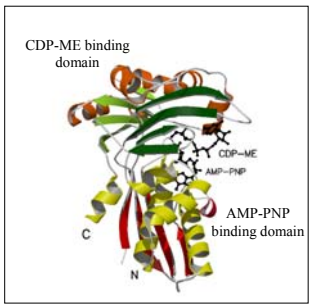
Lys10 and Asp141 participate in hydrogen bonding interactions to polarize the hydroxyl CDP-ME O2M. Thus, Asp141 acts as a general base to generate the O2M nucleophile and Lys10 contributes to the stabilization of the transition state. The binding of the cofactor places the γ-phosphate to accept the nucleophilic attack.



Results

The enzyme was crystallized in the presence of AMP-PNP which is a non-hydrolysable analogue of ATP and CDP-ME, the substrate.

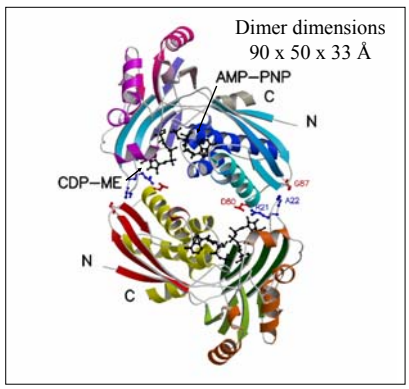
Resolution (Å)	2.00
Space group	$P2_1$
Unit cell	61.0 76.3 68.8 Å β=107.8 ° α=γ=90°
N° of reflections	39,761
Completeness	94.3
I/σ(I)	12.89
R merge (%)	4.8
Solvent content (%)	46.2
Number of Se site	12
Protein residues/atoms	566 / 4501
R-work (%)	16.6
R-free (%)	20.2
Ramachandran analysis	
- (%) favorable	92.9
- (%) additional	7.1



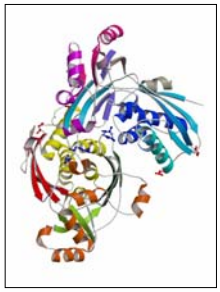
The overall structure displays the characteristic α/β two-domain fold of the GHMP kinase superfamily (**G**alactose **H**omoserine **M**evalonate and **P**hosphomevalonate) **[3]**.

The active site is located in deep cleft at the interface of the CDP-ME binding domain (or substrate binding domain) and the AMP-PNP binding domain (or cofactor binding domain). AMP-PNP is stabilized in a rare *syn* conformation

CDP-ME kinase is an homodimer that encloses a large solvent-filled channel with the two active sites located at each end.



Results of the cocrystallization with a CMP-kinase inhibitor



Structural information of a potential inhibitor bound to the enzyme could lead to support the development of novel broad spectrum antimicrobial drugs.

In this way we crystallised CDP-ME kinase with AMP-PNP and with AraCMP (1-β-d-arabinofuranosylcytosine monophosphaste). First analysis show that the overall structure is conserved, but the dimer formed is different in the presence of AraCMP.

We did a SAXS (**S**mall **A**ngle **X**-ray **S**cattering) experiment to check that the dimerisation differences are not due to a crystallisation artefact. The results are not concluant.

Conclusions and future work

Our crystallographic analysis of a ternary complex of CDP-ME kinase has delineated the structure, specificity, and mechanism of the enzyme responsible for phosphorylation in the nonmevalonate route of isoprenoids biosynthesis.

Structural information of an available drug bound to the CDP-ME kinase could lead to support the rapid development of novel broad spectrum antimicrobial drugs. The model of the CDP-ME kinase cocrystallised with AMP-PNP and AraCMP showed a dimerisation difference.

Experiments including fluorescence should elucidate the problem of the dimer difference and indicate a binding for the potential inhibitor.

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[1] Rohdich F., Eisenreich W., Wungsintaweekul J., Hecht S., schuhr C.A. and Bacher A., **The FEBS Journal**, 268, 3190-97 (2001). **[2]** Boucher Y. and Doolittle W. F., **Molecular Microbiology**, 37, 703-716 (2000). **[3]** Cheek S., Zhang H. and Grishin N.V., **Journal of Molecular Biology**, 320, 855-881 (2002).